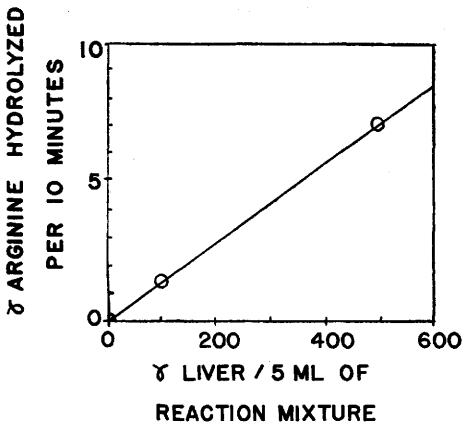

FIG. 4. Linear response of arginase activity *vs.* liver concentration (avg of 2 experiments).

system was calculated to be 1.53 x 10^{-4} M which agrees with a calculation of the Michaelis constant from data presented by Van Slyke and Archibald(6).

Summary. A method has been presented for the rapid determination of arginase with a minimum of operations and equipment. The activity of as low as 20 γ of whole liver can be determined. The arginine remaining after enzymatic hydrolysis is estimated by a quantitative modification(9) of the Sakaguchi reaction. Additional studies are presented indicating the necessary substrate concentration, the pH optimum, and linearity of the enzyme concentration curve for these experimental conditions.

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Effect of Cholinergic Drugs on Development of Chick Embryo.* (22320)

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There is sufficient evidence to indicate the presence of cholinesterase in embryos. Zacks (10) demonstrated the presence of cholinesterase in the embryonic chick from 0-96 hour stages. Wenger(8) followed the development of cholinesterase in spinal cords of chick embryos of 5½ to 11 days of incubation. Nachmansohn(4,5) determined cholinesterase activity in chick nerve and muscle at 6 or 9 days of age. He followed the increasing levels of activity of this enzyme to about the 15th day and showed a higher rate of increase until hatching. Similar observations have been made on early and later stages of amphibian embryos (Youngstrom(9), Sawyer (6,7), Boell and Shen(1,2)).

Since cholinesterase is present during early embryonic stages and increases steadily as the neuromuscular apparatus appears, the administration of enzyme inhibitors and the accumulation of acetylcholine could be attended by developmental abnormalities. To investigate this possibility, 4-day chick embryos were treated with various doses of cholinesterase inhibitors (prostigmine bromide; physostigmine salicylate), the enzyme

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TABLE I. Effects of Acetylcholine (Ac) and Atropine (A) on Development of Chick Embryos.

Drug	Route	Eggs	mg of drug or 0.85 cc NaCl at days of incubation								Mortality				Sac.	Wt (g)	Comments
			4	5	6	7	8	9	11	12	5	6	7	8	13		
Ac	A.s.	2	.5	.25								2					
		2	.5	.25	.5		.4	.4	.4	.4					2	8.5, 7	Viscera ectopic
		1	1.0	.50	.2		.2	.2	.2	.2					1	9	Normal
	Y.s.	2	.3	.3								2					
		2	.25	.25	.3		.25	.25	.25	.25					2	8.6, 9.3	Viscera ectopic
		1	.5	.5							1						
	E.m.	2	.2	.2		.2					1			1			
		1	.2	.2		.2	.2		.2	.2					1	9	Normal
A	Y.s.	1	.1	.1	.1		.1		.1	.1					1	8.9	
		4	.4	.4	.4							2	2				
	E.m.	2	.1	.1							1	1					
		3	.2								3						
		1	.2	.1	.2		.2	.2	.2	.2					1	8	Normal
		3	.5	.5	.1		.1	.1	.1	.1					3	7.5, 8.8, 8.9	"
S	E.m.	2	.2	.2	.2		.2	.2	.2	.2					2	9.8, 10	"
Cn		2													2	12, 12.5	"
Totals		31									6	7	2	1	15		

S, 0.85 saline controls; Cn, untreated embryos; Sac., time of sacrifice in days of incubation; A.s., air sac; Y.s., yolk sac; E.m., on the embryo.

substrate (acetylcholine) and a cholinergic blocking agent (atropine).

Method. Eggs containing vigorous 4-day embryos were obtained from a local hatchery. Various amounts of atropine, acetylcholine, eserine salicylate, and prostigmine bromide, were administered in either the yolk sac, air sac, or directly on the embryo. Since boric acid is used as a preservative in the prostigmine bromide preparation, control embryos were treated with 0.01% boric acid and 0.85% sodium chloride. Animals were sacrificed at 7-13 days of incubation, fixed in Bouin's or DeCastro's fluid and stained with Cajal silver and hematoxylin technics. Preparations with silver were used to examine the nervous system.

Results. Large amounts of acetylcholine (1.20 mg-2.85 mg) injected during the interval of 4-12 days of incubation were well tolerated as were similarly administered large doses of atropine (0.60 mg-1.50 mg). Of 27 embryos treated, 11 survived 13 days' incubation, and except for slight decreases in weight and ectopic viscera all were grossly of normal appearance (Table I).

During the interval of 4-8 days' incubation 42 embryos were treated with physostigmine bromide, eserine salicylate and a combination of physostigmine bromide and acetylcholine (Table II). The total amount of eserine or physostigmine given to each embryo was within the range of 1.0 mg-15.0 mg. Of the above 42 embryos, 22 survived 7-13 days' incubation and in all but 3 of the survivors, a remarkable kyphosis syndrome was observed (Fig. 1-5).

The syndrome as observed in 9-13-day embryos is briefly described (Fig. 1-5). The head was approximately the same size as controls, eyelids were retarded, the lower beak shortened, and the external auditory meatus connected with a fissure extending inferiorly and anteriorly. The meatus was larger than in controls. The neck and trunk were reduced approximately one half in length and this can be accounted for by 3 sharp compensating S-shaped curvatures in the cervical, thoraco-lumbar, and sacral-coccygeal vertebrae, respectively (Fig. 5). Bodies of the vertebrae were compressed and wedge-shaped at levels of sharp bending. Ankylosis of vertebrae was observed at levels where the vertebrae were compressed. Upper and lower extremities were shortened.

Throughout all levels of the spinal cord of

TABLE II. Injection of Prostigmine Bromide (P) and Eserine Salicylate (E) into Yolk Sac of Embryonic Chick and Effects on Development.

Drug	Eggs	Drug (mg) or 0.85% NaCl (cc)					Days of incubation: Mortality						Sacrificed						Sn	
		4	5	6	7	8	5	6	7	8	9	10	7	8	9	10	11	13	+	—
P	8	5.0						1	1				1	2	2	1			5	1
	6	5.0	5.0			5.0						1		1	2	1	1		5	
	6	.5	.5			.5			2					1	1	2			4	
P + Ac	6 P	.5			.5				1		2				1	2			3	
	Ac	.4			.4															
E	8	.5		.5		.5	2			2	1							3	1	2
	8	1.0					1	5	1							1			1	
B	8	.1	.5			.5		4					2		2					4
S	8	.1	.5			.5				2					6					6
Totals	58						3	10	5	4	3	1	3	4	14	7	1	3	19	13

S, 0.85% saline controls; B, 0.10% boric acid; Ac, acetylcholine; Sn, syndrome produced with prostigmine bromide or eserine salicylate (Fig. 4 and 5).

kyphotic animals, the extensive hypoplasia and atrophy of anterior horn cells was most striking. A reduction in volume of musculature was observed along the vertebral column and in the lower extremity. Spinal and sympathetic ganglia were well differentiated but often fused into continuous masses in regions of severe bending. Pedicles and laminae of sacral and coccygeal vertebrae were usually missing. This was generally associated with absence of the spinal cord. Although the brain and brain stem were reduced in size, no gross abnormalities were observed.

Above experiments were supplemented with observations on 12 embryos injected on the fourth day with .05 mg of physostigmine salicylate. Nine survived 8-12 days' incubation. Of these, 2 were normal, 4 with slight kyphosis at thoracic and lumbar levels; one with normal vertebral column but with shortened lower beak, retarded eyelids and micromelia (Fig. 6); one with no coccyx and with shortened extremities. With high dosages it was typical to find abnormalities in combination with kyphosis. When weaker dosages (.05 mg) were given, the abnormalities were scattered, appearing singly or in unpredictable combinations.

Discussion. Landauer(3) reported the morphological consequences of treatment with sulfanilamide, eserine, and insulin. With heavy doses of these drugs, shortened beaks, micromelia, syndactylism, clubbed down and microphthalmia were observed. Weaker doses produced abnormalities of less degree and a scattering of abnormalities, observed singly or in various combinations. His results were partly confirmed with our observations on embryos treated with weak doses of prostigmine bromide or physostigmine salicylate (Fig. 6). Since similar abnormalities were reported in embryos treated with sulfanilamide and insulin, the mechanism indicated is probably of a non-specific nature.

Observations were made by Sawyer(6) on amblystoma embryos reared in various concentrations of eserine (0.01%-0.001%). At Harrison stage 46 a majority of eserinized animals developed one or 2 sharp curvatures of the trunk. There was little retardation of growth, digits were normal, length of gills and height of dorsal fin were decreased. The width of the head was increased. Curvatures of the trunk did not disappear after decapitation or destruction of the spinal cord. This was interpreted as evidence of the muscular origin of the bend reaction. Removal of animals from the inhibitor solution and transfer to water did not eliminate the bend reaction. This persisted as though the skeleton had become fixed in position. Chick embryos eserinized at 4-8 days of incubation subsequently developed extensive kyphosis observed at 7 to 13 days' total incubation. Although this condition seemed more severe than that described for amblystoma by Sawyer(6), the mechan-

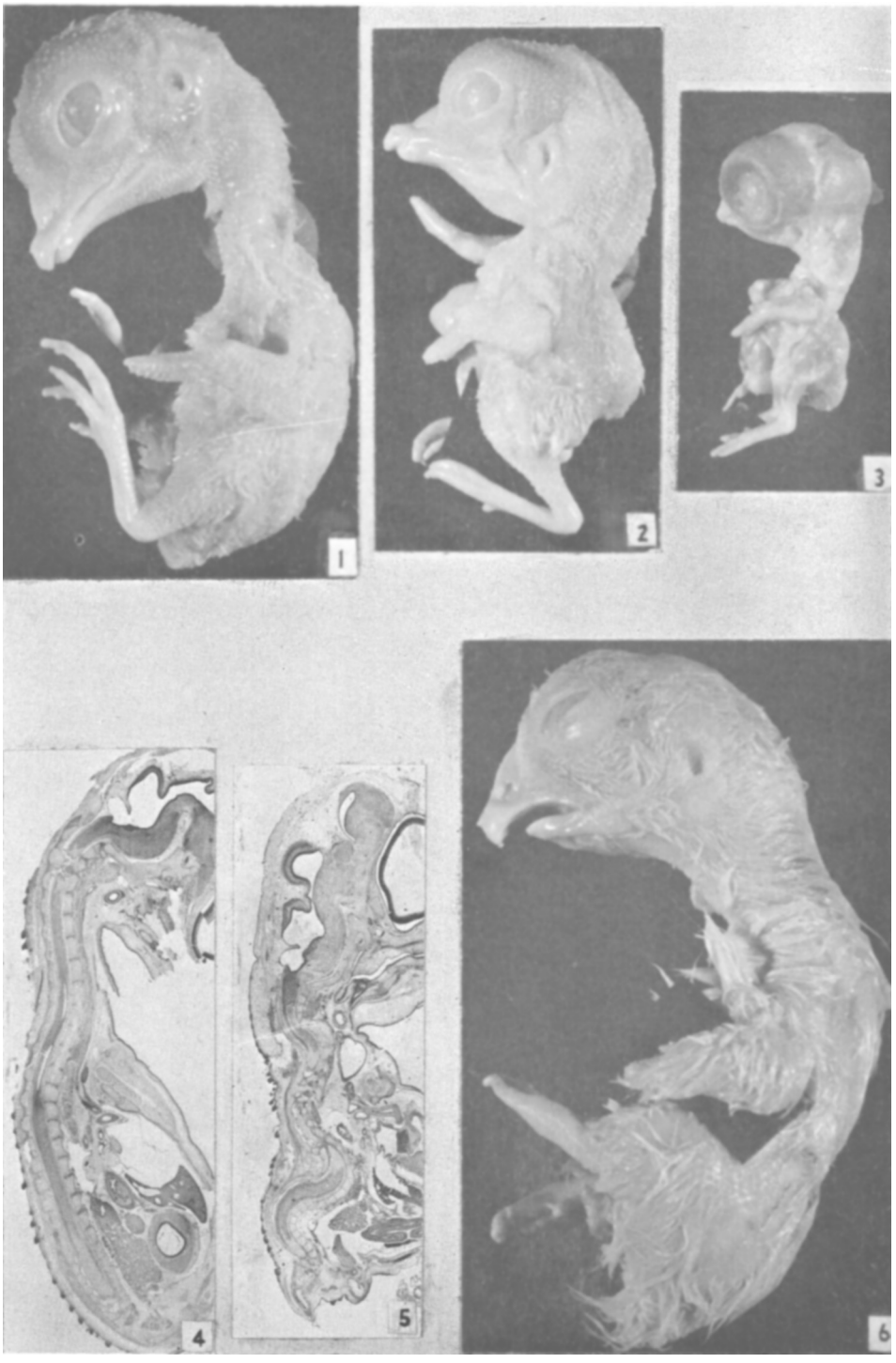

FIG. 1. Control embryo (13-d) treated (yolk sac) on 4th, 5th and 8th days of incubation with 0.1, 0.5 and 0.5 cc of 0.85% saline. ×2.

FIG. 2. Embryo (P6, 13-d) treated (yolk sac) on 4th, 5th and 8th days of incubation with 5 mg of prostigmine bromide; total dosage 15 mg. ×2.

FIG. 3. Embryo (P1, 8-d) treated (yolk sac) on 4th day of incubation with 5 mg of prostigmine bromide. ×2.

FIG. 4. Mid-sagittal section of control embryo (12-d) treated (yolk sac) on 4th, 5th and 8th days of incubation with 0.1, 0.5 and 0.5 cc of 0.85% saline. ×3.4.

FIG. 5. Mid-sagittal section of embryo (12-d) with extensive kyphosis showing 3 compensating S-shaped curvatures. Treated (yolk sac) on 4th, 5th and 8th days of incubation with 5 mg of prostigmine bromide; total dosage 15 mg. ×3.4.

FIG. 6. Embryo with micromelia and shortened lower beak (E-10, 13½-d). Treated (yolk sac) on 4th day of incubation with 0.05 mg of physostigmine salicylate. ×2.

ism of formation may be similar.

It is interesting that the treatment of embryos with large doses of acetylcholine during 4 to 12 days of incubation did not result in malformations similar to those obtained with cholinesterase inhibition. This would suggest the destruction of acetylcholine shortly after its administration. Since cholinesterase increases rapidly as the neuromuscular system develops this is entirely possible. Atropine, a cholinergic block, had no deleterious effect on development.

Summary. 1. Large doses of acetylcholine (1.20-2.85 mg) were injected in the yolk sac, air chamber, or directly on the embryo during the 4-12-day incubation period. These doses were well tolerated by the embryo and attended with no gross abnormalities. Atropine similarly administered produced no malformation. 2. The yolk sac of 4-day embryos was injected with weak doses (0.05 mg) of physostigmine salicylate. Shortened beaks, micromelia, and retarded development of eyelids were observed at 8 to 13 days' incubation. Inasmuch as similar anomalies were reported in sulfanilamide and insulin treated animals, these anomalies cannot be considered eserine specific. 3. With larger doses (1-15 mg) of prostigmine bromide or physostigmine salicylate, the above mentioned anomalies were generally associated with severe midline bendings of the vertebral column (Figs. 4,5), and this was interpreted as specific for cholinesterase inhibition.

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Extraction and Concentration of Thromboplastic Material from Human Urine. (22321)

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Although human urine is known to have thromboplastic property(1,2), little attention has been given to the concentration or purification of the urinary component responsible for this property. There is described herein a simple method for extraction and concentration from normal urine of material with thromboplastic activity, and some characteristics of this material are discussed.

Material and methods. Pooled urine obtained without preservative from normal men is used. The urine, refrigerated not longer

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